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Determination of pyrethroid pesticide residues in vegetables by pressurized capillary electrochromatography

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Abstract

A simple and rapid pressurized isocratic capillary electrochromatography (pCEC) method has been developed to separate six pyrethroid pesticides. The effects of pH of buffer, organic solvent content, buffer concentrations and applied voltage on the separation of six pyrethroids were investigated. Under the optimized conditions, the pCEC method developed allows baseline separation of a complex mixture of six pyrethroids in <20 min. The method is applied to the analysis of these pesticide residues in Chinese cabbage. The limits of quantification (LOQ) ranged from 0.5 to 0.8 μg/ml (corresponding to 0.05 and 0.08 mg/kg in the vegetable sample), with relative standard deviations (R.S.D.) <5.0%. Mean values of recoveries for six pyrethroids ranged from 89.6 to 96.3%, respectively.

Keywords: Pyrethroid pesticides; Residue; Pressurized capillary electrochromatography; Vegetable

1. Introduction

Synthetic pyrethroids are widely used as the broad-spectrum pest control agents in agricultural production because of their selective insecticidal activity, rapid biotransformation and excretion by the mammalian catabolic system and their non-persistence in the environment [1–3]. However, the risk of pesticide residues remaining on the food consumed is present at one time, which is due to the overuse and accumulation in food chain. To protect consumers from unsafe level, the Codex Alimentarius Commission of the United Nation's Food and Agriculture Organization and the World Health Organization has established maximal residue limit (MRL) for pesticides over a variety of foods [4]. A rapid and simple analytical method as well as high accuracy in the identification and quantitation of the pyrethroid residues is necessary to monitor and regulate their usage for ensuring food quality and safety.

Almost all the analytical methods for the determination of pyrethroid residues in agricultural commodities are based on the use of chromatographic techniques, mainly gas chromatography (GC). GC with an electron capture detector (ECD) [5–9] is the most popular method, while GC–MS is used for pyrethroid residues confirmation [10–13]. Thin layer chromatography (TLC) detection using chromatographic reagent [14] is less widely used than GC and HPLC because of low detection limit. Some applications of HPLC to the analysis of pyrethroid residues in fruits and vegetables have also been reported [15–17]. In this instance, since the selected pyrethroid differ widely in polarity, a gradient elution mode has to be used for their separation. Moreover, the large amounts of toxic organic solvents and pesticides used in experiment create some problems. To minimize solvent consumption and improve the column efficiency, capillary electroseparation methods, for example, Micellar electrokinetic chromatography (MEKC) have been performed to analyze neutral and charged species [18,19]. However, MEKC does not appear to be a useful complement to HPLC to separate very hydrophobic neutral analytes, such as pyrethroid pesticides.

Capillary electrochromatography (CEC) has become a powerful separation technique for both neutral and charged compounds because it couples the high efficiency of CZE and high selectivity of HPLC [20–23]. Several groups [24,25] have reported the separation of pyrethrins and pyrethroids by CEC. At present, CEC is not yet mature enough and face with some practical difficulties, such as bubbles formation and column dry-out during experiment, which hold back the progress of this

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technique [26,27]. Supplementary pressure can be applied to a column in CEC by using an micro-HPLC pump (or pumps) to overcome these problems. This technique is known as pressurized capillary electrochromatography (pCEC) [28,29]. To date, there have been no report attempts to separate and quantify pyrethroid pesticides using pCEC.

In this study, pCEC has been used to separate six pyrethroids. Several electrochromatographic parameters, such as pH of buffer, organic solvent content, buffer concentrations and applied voltage were evaluated to optimize the pCEC separation pyrethroids. The optimized method was shown to be effective in separating and quantifying the pyrethroid pesticides in Chinese cabbage.

2. Experimental

2.1. Instrumentation

pCEC was carried out on a Trisep™ 2010GV CEC system (Unimicro Technologies, Pleasanton, CA, USA) which comprised a solvent gradient delivery module, a high-voltage power supply (+30 and −30 kV), a variable wavelength UV–vis detector, a micro fluid manipulation module (including a six-port injector) and a data acquisition module [30]. A high-pressure syringe pump was used to provide supplementary flow to the CEC column. The mobile phase is driven by electroosmotic flow (EOF), as well as pressurized flow and enters into six-port injection valve. Samples injected are delivered to the injection valve and introduced in the internal 2 μl sample loop, and then be carried to the four-port split valve by the mobile-phase flow. After splitting in a four-port valve, the flow enters a capillary column under constant pressure controlled by a backpressure regulator. A negative voltage was applied to the outlet of column, and the inlet of column was connected to the split valve and grounded. In this experiment, the isocratic elution system was used and 225 nm was used as the measurement wavelength of the UV–vis detector. FS-1 Hi-speed homogenizer (Jintan Fuhua Instrument Corporation, Jiangsu, China) and BF2000 nitrogen evaporator (Beijing Bafang Century Science-Tech Corporation, Beijing, China) were also used.

2.2. Materials

Capillary column of 200 mm × 100 μm i.d. × 375 μm o.d. packed with 3 μm ODS particles was supplied from Unimicro Technologies. Buffer solutions were prepared using Tris (AR) (Chemical Reagent Corporation, Shanghai, China). Acetonitrile (ACN) obtained from Luzhong Chemical Reagent Corporation (Shanghai, China) was HPLC-grade. The pyrethroid pesticides (see Fig. 1) were purchased from ChemService (West Chester, PA, USA). Hexane, acetone, acetonitrile, sodium chloride (Shanghai Chemical Reagents Corporation, Shanghai, China) for pesticide residue analysis were all analytical reagent-grade, deionized water was obtained from a Milli-Q water purification system (Millipore, France). Accubond II florisil cartridges, 1 g/6 ml (Agilent Technologies, UK) was used for clean-up vegetable samples.

2.3. Preparation of standards and fortified samples

Standard stock solutions were prepared in ACN to give a final concentration of 1.0 mg/ml. Working solutions were freshly prepared by diluting standard stock solutions with ACN. All solutions were stored in a refrigerator at 4 °C and protected from light. For preparation of fortified samples, certain volume of standard stock solution (1.0 mg/ml) were added to 50 g of

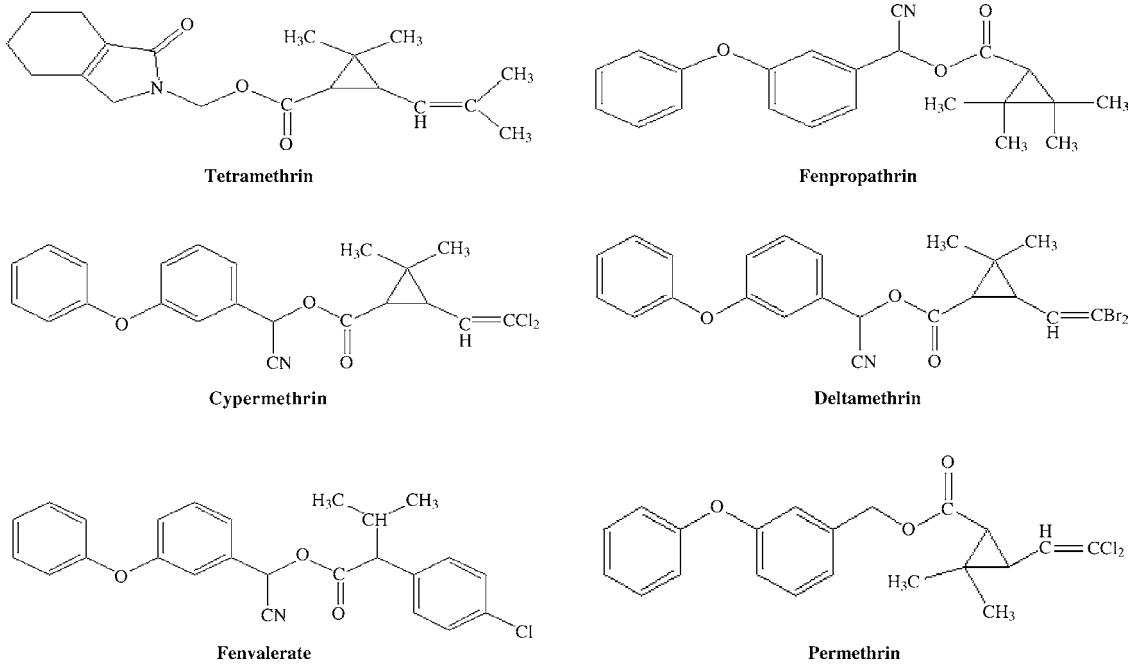

Fig. 1. Chemical structures of six pyrethroids.

homogeneously chopped vegetable samples, and produce the samples with spiked analytes of 0.1 and 0.5 mg/kg, respectively (equivalent to 1.0 and 5.0 μg/ml in the final extract). The fortified samples were allowed to stand for a few minutes before extraction to allow the spike solution to penetrate the test material.

2.4. Procedure for sample preparation

2.4.1. Extraction procedure

Fifty grams of chopped vegetable sample was placed into a homogenizer jar and mixed with 100 ml of ACN. Then the mixture was homogenized for 3 min at high speed. The extract was filtered through a 12 cm Buchner funnel. Rinsed the homogenizer jar with two 25 ml portions of ACN and used the washes to rinse the residues in the Buchner funnel. Transferred the filtrate to a 500 ml separating funnel, added 15 g of sodium chloride and mixed vigorously for 60 s. Allowed layers to separate and discard aqueous layer. Evaporated to nearly dryness on a rotary evaporator at 80 °C and allowed to evaporate the remaining solvent with a nitrogen evaporator.

2.4.2. Clean-up procedure

Dissolved the residue obtained from the extract in 5 ml of hexane. Preconditioned a florisil SPE column with 5 ml hexane:acetone 90:10 (v/v) and 5 ml hexane. Transferred 1 ml of the sample extract (equivalent to 10 g Chinese cabbage) to the column and allowed the level to go down until just above the florisil packing. Eluted pyrethroid residues with 5 ml of hexane:acetone 90:10 (v/v), and collected eluate in a 10 ml spherical flask. The eluate was concentrated to nearly dryness on a rotary evaporator at 50 °C, and the remaining solvent was allowed to evaporate with a nitrogen evaporator. The obtained residue was dissolved in 1 ml ACN and then filtered through 0.22 μm micro filter. The final extract contained 10 g of Chinese cabbage per 1 ml.

2.5. pCEC procedure

All standard and fortified sample solutions were passed through a 0.45 μm filter before use. Mobile-phase solution was degassed in an ultrasonic bath for 20 min before use. A supplementary pressure (500 psi) was applied to the column inlet during the separation. The flow rate of the pump was 0.05 ml/min. Before pCEC experiments, the column was conditioned on the instrument with the mobile phase for 1 h, applied voltage was first ramped from 0 to −10 kV and then operated at −10 kV. The column was equilibrated for about 30 min after the mobile phase was changed and the temperature of the column was kept at room temperature.

3. Results and discussion

For all analytes, the sum of isomer peak area was taken for quantification. Although the majority of the selected pyrethroids show maximum absorbance in the low UV region (<225 nm), 225 nm was used due to the large interfering peaks showed up when lower wavelengths were selected.

The separation mechanism in pCEC for neutral compounds is mainly based on the differences in partition of the sample components between the mobile and stationary phases. Whereas the differences in electrophoretic mobility of components also make a contribution to the separation mechanism for charged species. Since the driving factor in the separation of neutral solutes, such as the present pyrethroids by pCEC is determined by both EOF and pressure flow, an investigation of the effects of several parameters (such as pH of buffer, organic solvent content, buffer concentrations and applied voltage) on the retention and selectivity is necessary for optimal performance. Optimization of the separation conditions was achieved through testing the retention behavior of pyrethroid mixtures on a commercial micro-packed porous ODS column.

3.1. Effect of organic solvent content

A two-component mobile phase consisting of ACN and buffer was used in this work. Consequently, we conducted a series of experiments to optimize the separation of six pyrethroids using various volume fractions of ACN while keeping the phosphates buffer concentration of 5 mmol/l (pH 8) and an applied voltage of −10 kV. Fig. 2 illustrates the effects of various percentages of ACN on the separation of six pyrethroids. In this study, the EOF decreases with the decrease of ACN content. Conversely, the migration times and selectivity factors increase with the decrease of ACN content, due to changes in the partitioning of the hydrophobic analytes between the stationary and mobile phase. The neutral pyrethroids are separated primarily on the basis of differential partitioning into the alkyl-bonded phase. In the experiment, good separation can be obtained using a mobile phase containing lower concentration of organic solvent, which promotes hydrophobic interactions between the analytes and stationary phase. Taking into consideration the resolution and speed of analysis, 60% ACN was used to further optimize separation conditions for the mixture of six pyrethroids.

3.2. Effect of pH of buffer

To investigate the effect of pH on migration behavior, different pH varied from 6 to 9 (pH 6–9) were used for pCEC analysis in mobile phase consisting of 60% ACN and 5 mmol/l phosphates buffers. In this instance, there was no noticeable increase in resolutions, that indicated the chromatographic partition account for the separation because all the analytes is neutral over the pH range examined. At the same time, the retention times decreased. The velocity of EOF is known to be dependent on the pH of the mobile phase due to its effect on the extent of dissociation of surface silanol groups [31]. Generally, at constant ionic strength, increasing the pH results in an increase in EOF and therefore shorter migration times of the analytes. To increase the velocity of EOF and avoid the risk of the dissolution of the silica bed, pH 8 was chosen in the following experiment. Compared with phosphate buffer, Tris–HCl buffer was with lower conductivity and chosen in further experiments [32].

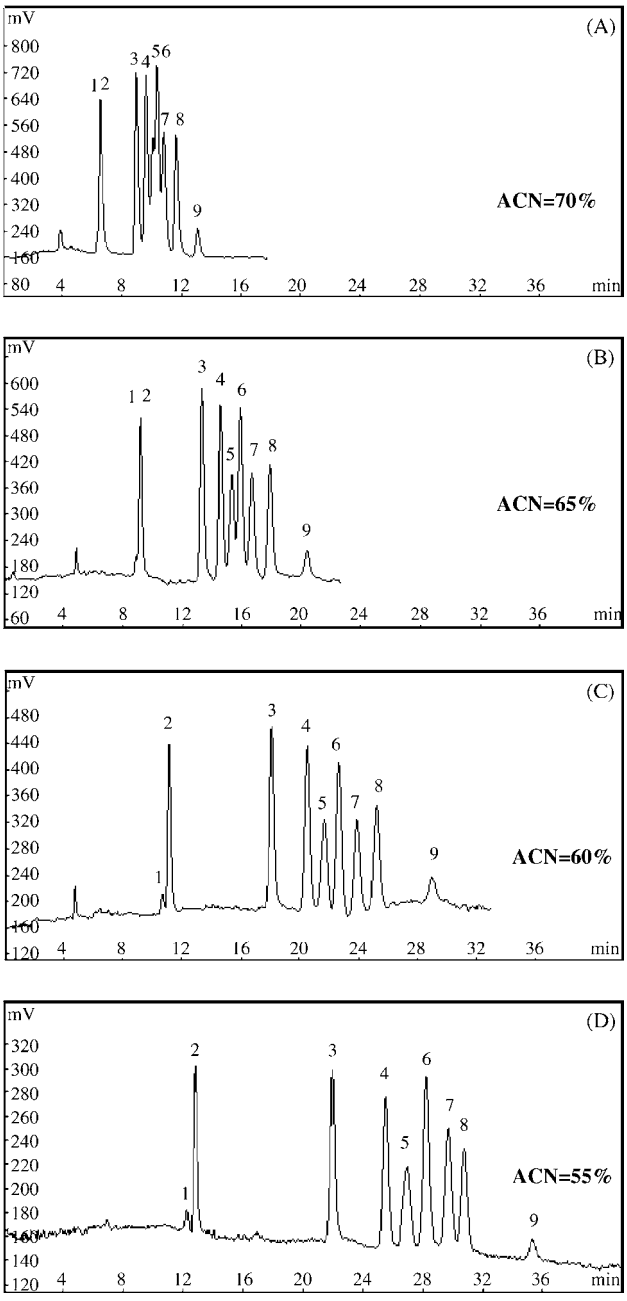

Fig. 2. Effect of ACN percentage on the separation of six pyrethroid standard samples. Conditions—mobile phase: ACN-5 mmol/l phosphate buffer, pH 8, −10 kV applied voltage, flow rate 0.05 ml/min; peaks: 1 and 2, tetramethrin; 3, fenpropathrin; 4 and 5, cypermethrin; 6, deltamethrin; 7, fenvelerate; 8 and 9, permethrin.

3.3. Effect of Tris buffer concentration

The effect of Tris concentration on the separation of six pyrethroids was studied using 5, 10 and 20 mmol/l of Tris at pH 8. With increasing Tris concentration, retention times of the analytes increased, which is due to the change of salt concentrations buffered alter the double layer on the silica surface and finally reduce the EOF [33]. Higher Tris concentration (>10 mmol/l) was not feasible for longer retention times, which in turn leads to band broadening. Thus, a mobile phase of 10 mmol/l Tris was chosen in all subsequent experiments.

3.4. Effect of applied voltage

The effect of the applied voltage on the efficiency, resolution and analysis time of the six pyrethroids was determined in a mobile phase containing 60% (v/v) ACN, 10 mmol/l Tris buffer (pH 8) at constant pressure. The applied voltage was varied from −10 to −18 kV. As expected, a higher voltage reduced the retention times due to an increase of EOF. At −18 kV, the resolution between peaks 5 and 6 is poor. In contrast, longer analysis time was needed for the separation of the mixture at −10 kV. The best separation with a short analysis time was obtained using −15 kV. The separation profile obtained under these optimal conditions is similar to those reported for HPLC, but with significant improvement in resolution, analysis time for the pyrethroids studied. Using a column >20 cm could increase resolution of the studied pyrethroids. The pCEC analysis is completed within 20 min under isocratic conditions, whereas a 30 min gradient elution would be required in HPLC to obtain a similar separation pattern for the same mixture [15].

3.5. Separation and quantification of pyrethroid extracts

3.5.1. Calibration curves, the limits of quantification (LOQ), limits of detection (LOD) and reproducibility

Under the optimized conditions (mobile phase; 40%, v/v of 10 mmol/l Tris; pH 8 and 60%, v/v ACN; applied voltage, −15 kV; detection wavelength, 225 nm; supplementary pressure, 500 psi; flow rate, 0.05 ml/min), the electrochromatograms for the mixture of six pyrethroids is shown in Fig. 3. It can be seen that six analytes were baseline-separated within 20 min. Analytical figures of merit are given in Table 1 when using an external standard method and solvent standards for calibration. The LOQ for the pyrethroid pesticides were calculated using the lowest concentration where relative standard deviations (R.S.D.%) is estimated to be <5% [34]. The LOD, which was defined as the concentration at which the signal-to-noise ratio is 3, were determined by analysis of standard samples of gradually decreasing concentration. The short- and long-term reproducibility of the retention time, peak area for some representative peaks are presented in Table 2. As expected, the R.S.D. for pyrethroids were

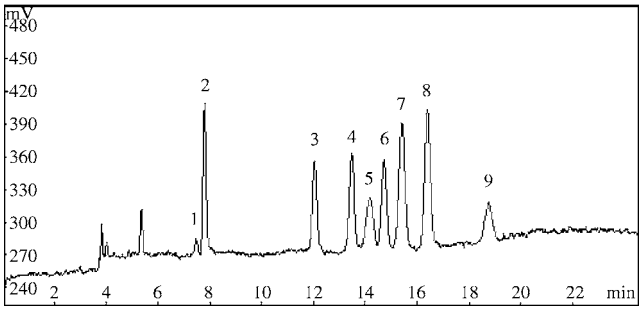

Fig. 3. Typical electrochromatograms for six pyrethroids obtained under the optimal conditions. Conditions—mobile phase: ACN-10 mmol/l Tris (60:40; pH 8), −15 kV applied voltage, flow rate 0.05 ml/min, solutes as Fig. 2.

Table 1
Quantification parameters of the proposed method

Compound	Regression equation	Correlation coefficient	Liner range (μg/ml)	LOQ (μg/ml)[a]	LOD (μg/ml)[b]
Tetramethrin	$y = 0.1207x + 0.1085$	0.9992	0.5–50.0	0.5	0.2
Fenpropathrin	$y = 0.1182x + 0.0699$	0.9995	0.5–50.0	0.5	0.2
Cypermethrin	$y = 0.1509x + 0.0242$	0.9994	0.5–50.0	0.5	0.2
Deltamethrin	$y = 0.0566x + 0.2572$	0.9991	0.8–50.0	0.8	0.3
Fenvalerate	$y = 0.0514x + 0.0314$	0.9993	0.8–50.0	0.8	0.3
Permethrin	$y = 0.0978x + 0.0047$	0.9902	0.8–50.0	0.8	0.3

[a] Based on the lowest concentration where the R.S.D. (%) is estimated to be <5%.
[b] Based on the S/N = 3.

Table 2
Retention time and peak area reproducibilities[a]

Compound	t_R (min)	R.S.D. (%)	Peak area	R.S.D. (%)
Run-to-run ($n = 7$)				
Tetramethrin (1)	7.02	0.08	64863	1.82
Fenpropathrin (3)	11.69	0.07	599629	1.32
Cypermethrin (4)	13.17	0.13	802161	1.23
Deltamethrin (6)	14.43	0.15	228681	1.46
Fenvalerate (7)	15.09	0.09	151802	1.61
Permethrin (8)	16.13	0.10	287774	1.54
Day-to-day ($n = 7$)				
Tetramethrin (1)	7.00	0.72	62493	4.26
Fenpropathrin (3)	11.68	0.85	570811	3.12
Cypermethrin (4)	13.15	0.78	839090	3.65
Deltamethrin (6)	14.41	0.89	213962	4.52
Fenvalerate (7)	15.08	0.76	161574	4.96
Permethrin (8)	16.11	1.02	294772	4.85

[a] Conditions identical to Fig. 3.

generally low from run-to-run on the same day, when compared to typical day-to-day variations, and the intra- and inter-day precisions of retention times and peak area are <5%.

3.5.2. Spike recoveries

In order to examine the reliability of the method, the recoveries of six compounds were investigated. To determine recoveries, Chinese cabbage samples were spiked with 0.1 and 0.5 mg/kg of each standard solution of pyrethroid, then extracted and analyzed as described in Section 2.4. From results showed in Table 3, it can be seen that recoveries were between 89.6 and 96.3%.

3.5.3. Analysis of pyrethroid residues in Chinese cabbage

The proposed method was applied to the determination of pyrethroid residues in Chinese cabbage, samples was done as described in Section 2.4. As can be seen from Fig. 4, this method

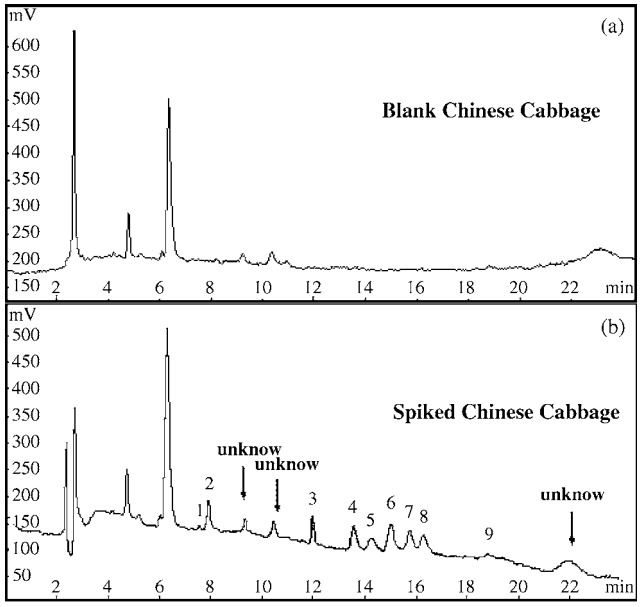

Fig. 4. Electrochromatograms of (a) a blank Chinese cabbage extract compared with (b) a Chinese cabbage extract spiked with 2.0 μg/ml of tetramethrin, fenpropathrin, cypermethrin, deltamethrin, fenvelerate, permethrin, conditions same as Fig. 3.

was effective in eliminating the matrix interferences in real sample. MRL established by the Agricultural Ministry of China for the pyrethroid pesticides in Chinese cabbage are 0.5 mg/kg (in extract 5.0 μg/ml) for fenvalerate, deltamethrin, fenpropathrin; 1.0 mg/kg (in extract 10 μg/ml) for cypermethrin, permethrin. In all cases, the LOQs obtained in the experimental conditions are lower than MRL for these pyrethroids. By using solid-phase extraction, the described pCEC multi-residue method is sensitive enough to achieve these quantification levels for the determination of pyrethroid residues in Chinese cabbage samples. It is useful for the determination of low level of six pyrethroid pes-

Table 3
Mean recoveries of six pyrethroids from Chinese cabbage samples spiked at two levels of standard mixtures

Compound	Spiking level 1 (mg/kg)	Recovery (%)	R.S.D. (%) ($n = 6$)	Spiking level 2 (mg/kg)	Recovery (%)	R.S.D. (%) ($n = 6$)
Tetramethrin	0.1	91.2	3.2	0.5	96.3	2.1
Fenpropathrin	0.1	92.6	4.5	0.5	94.3	2.3
Cypermethrin	0.1	94.3	2.1	0.5	93.4	1.3
Deltamethrin	0.1	90.3	3.4	0.5	94.5	1.6
Fenvalerate	0.1	91.2	5.2	0.5	92.1	2.5
Permethrin	0.1	89.6	4.9	0.5	92.3	1.9

ticide residues in Chinese cabbage sample without obviously interference.

4. Conclusions

This work has demonstrated the development of a pCEC method for the efficient separation of the mixture of six pyrethroids. Under optimized conditions, baseline resolution of the six pyrethroids was achieved in <20 min. It was found that the reproducibility of the pCEC separation of six pyrethroids was increased dramatically when supplementary pressure was applied. The proposed method was successfully applied to analysis of real Chinese cabbage samples by using solid-phase extraction. Compared with other separation techniques, it is a more rapid, simple and effective method for separation and determination of six pyrethroid residues.

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